

## SEED COAT ANATOMY AND TAXONOMY IN EUCALYPTUS. III.\*

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(Plate iii; Text-figures 40-47.)

[Read 29th March, 1961.]

*Synopsis.*

*Series Miniatae*: The two species, *E. miniata* and *E. phoenicea*, which alone comprise the Series, have a number of points in common. These are the hemitropous seed without a raphe, an obsolete or missing cuticle, the suberized inner integument, no chalaza cork and the high number (up to six or seven or even nine) of small vascular bundles arranged in a semicircle along the upper chalaza border. They differ with regard to the sclerification of the epidermis, suberization of the hilum and occurrence of sclereid and crystal idioblasts.

*Series Tetrapterae*: The common features of *E. tetraptera*, *E. forrestiana* and *E. erythrandra* are: seed hemitropous with short raphe, outer cuticle missing, inner integument not suberized, nucellus cutinized. *E. steedmanii* does not fit into this Series because of its entirely different testa structure.

*Series Clavigerae*: This Series as formulated by Blake is a natural one. Seed hemitropous without raphe, strongly compressed dorsiventrally and thin, orbicular to ovate in outline. Outer cuticle well preserved, inner integument not suberized but partly resorbed and vascular system of various patterns. The embryo cotyledons have oxalate druses only in the large aleurone grains of the epidermis, sharing this particular location with all *Corymbosae* and species of *Angophora*.

*Series Corymbosae*: The seed is hemitropous and without a raphe, being either flattened dorsiventrally and wingless (Subseries *Ochrophloiae* and *Maculatae*) or laterally with a long terminal wing which is missing in very few cases (Subseries *Setosae*, *Gamophyllae*, *Eucorymbosae* and *Neocorymbosae*). *E. trachyphloia* with wingless seed compressed dorsiventrally would better be placed on this basis in the *Ochrophloiae* instead of the *Neocorymbosae*. The outer integument is multilayered with the cuticle well preserved, the inner integument not suberized but partly resorbed. Oxalate druses are present in the cotyledons as in the *Clavigerae*.

## Series MINIATAE Blakely.

The seed is hemitropous, dorsiventrally compressed and smooth in *E. phoenicea* (resembling *E. baileyana* and its relatives) and with strong ribs in *E. miniata* (similar to *E. erythrocoris*).

The *outer integument* is mostly multilayered on the ventral side and predominantly two-layered on the back; the outer cuticle is faintly discernible or missing.† The cells of the outer epidermis of *E. miniata* have thick anticlinal walls, the thickenings sometimes hourglass-like (Fig. 40b) with cutinized middle lamellae. In ribs and hilum rim they are perpendicularly elongated, some of them having periclinal divisions. In contrast, the epidermal walls of *E. phoenicea* are thin, though sclereids (with horse-shoe shaped thickenings) may occur here too, mainly restricted to the hilar and micropylar region (Fig. 40a). The sclereids in both species are lignified and heavily impregnated with tannins. There are sclereid idioblasts in the integumentary parenchyma on the ventral side of *E. miniata*, mostly close to the crystal layer and in radial alignment with the chalaza. The inner epidermis is a typical crystal layer, the cells with a thick bottom (the morphological outer wall) accommodating small crystals ensheathed by a cellulose

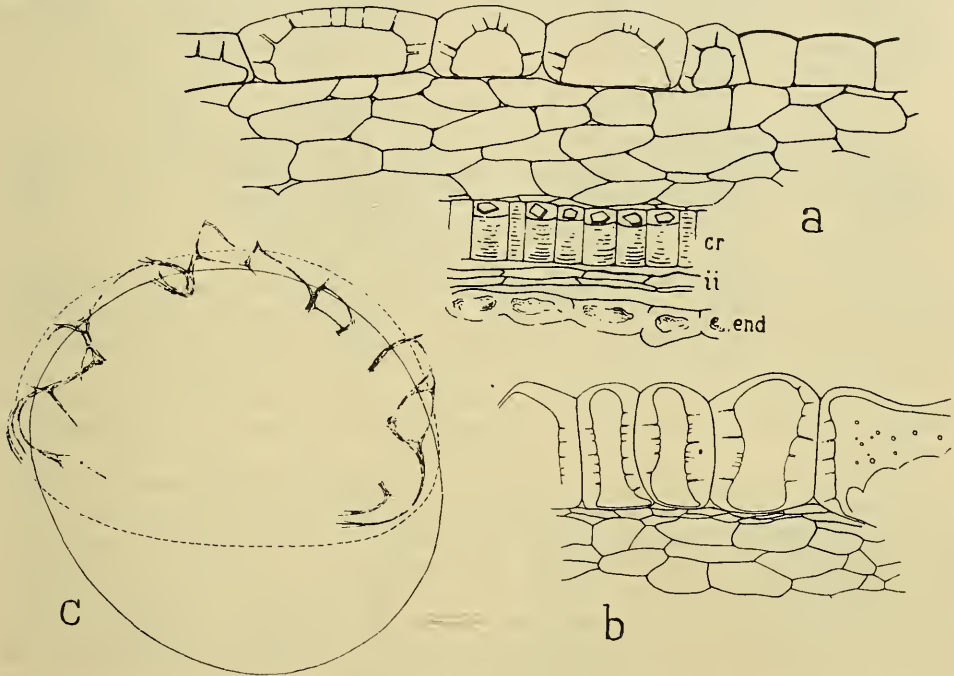
\* See Part I, PROC. LINN. SOC. N.S.W., 1958, 83, Part 1: 20-32; and Part II, *ibidem*, 1959, 84, Part 2: 278-291.

† In *E. phoenicea* minute patches of a very thin "crust" can be discerned on the surface. Its chemical nature will be examined later in connection with similar occurrences in other species.

membrane. In most of the *E. phoenicea* seeds the crystal layer at the rim of the chalaza is in-folded against itself so that in surface view (best examined from inside) the double layer of crystal-bearing cells forms a "collar" of variable width around the chalaza, as illustrated in Figure 45a for *E. haematoxylon*. In *E. miniata* lignified sclereid idioblasts were occasionally observed, intercalated between the somewhat smaller non-lignified cells of the crystal layer.

The *inner integument* is suberized and projects beyond the rim of the crystal layer into the "window", this projection being of variable width with a smooth, wavy or jagged contour.\*

*Chalaza.* The parenchymatous tissue within the gap in the crystal layer and inner integument is neither suberized nor lignified, therefore the so-called "chalaza cork" is (as in *E. tetradonta*) missing. Crystal-bearing idioblasts with uneven, often reticulate thickenings accommodating one or two ensheathed crystals per cell were seen in the centre of the chalaza of *E. miniata* only.



Text-fig. 40a-c.—a, b, trans. sect. through the outer integument showing sclerified epidermal cells: a, *E. phoenicea*, horse-shoe shaped thickenings in the micropylar region, 350 $\times$ ; b, *E. miniata*, some thickenings hour-glass-like, 230 $\times$ . c, *E. miniata*, vascularization pattern, 23 $\times$ . The dotted line limits the chalaza region, the solid line the hilum.

*Hilum.* It overlaps to a great extent (sometimes completely) the chalaza (Fig. 40c), is sharply circumscribed by a raised rim of sclereids in *E. miniata*, whereas in *E. phoenicea* sclereids may occur in more or less dispersed groups (very rarely confluent to form a complete sclerified frame) around the hilum. In *E. miniata* about two superficial layers are lignified and suberized. The occurrence of cells characteristically arranged in groups of four indicates that cell divisions were still in progress when suberization took place (Pl. iii, fig. 3). *E. miniata* is a further case of metaplasia by suberization of the hilum, at least *pro parte*, because only that part of it which overlaps the chalaza is

\* These projections are particularly prominent in *E. phoenicea* and they are the reason why in sections through the chalaza close to its periphery the inner integument may be seen as a continuous layer separating the chalazal tissue from the nucellar.

completely suberized while in the remaining part not all cells have suberin lamellae. In *E. phoenicea* no such suberization is encountered and the protection of the scar is dependent solely on deposits of tanniniferous substances.

*Vascularization.* The high number of small bundles entering the chalaza is characteristic for both species: up to 6 in *E. phoenicea*, up to 9 in *E. miniata*. The more of them present the more tender and shorter they are. They are arranged in a semicircle, their entrance close to the upper border of the hilum (Fig. 40c).

These two species are considered—on grounds other than seed structure—as closely related and the broader features of seed coat anatomy are in accord with this view. The anatomical discrepancies in such characters as epidermis structure, occurrence of sclereid and crystal idioblasts and suberization of the hilum suggest these features must be used with caution for taxonomic purposes.

S. T. Blake (p. 335) advocates grouping together of the Miniatae and *E. baileyana* and its relatives. However, the testa structure does not help to substantiate this suggestion. Nevertheless as far as our investigations have gone these two species show no closer affinity with any other groups of species so far examined and any hint of closer affinity from seed coat anatomy must await the completion of the study of the whole genus.

#### Series TETRAPTERAE Blakely.

In this Series Blakely lists in the 1935 edition of "A Key to the Eucalypts" only *E. tetraptera*, but in an addendum to the description of *E. steedmanii* he says, "I have proposed a new Series—Tetrapterae, for the reception of the three species . . .", which he implies are *E. tetraptera*, *E. forrestiana* and *E. steedmanii*.

The editors of the second edition, 1955, place *E. erythrandra* also in the Series Tetrapterae in accordance with the notes accompanying the description of this species, but in spite of Blakely's notes concerning *E. forrestiana* under *E. steedmanii* which they include in the Tetrapterae in this edition, they still retain *E. forrestiana* in the Series Contortae where it was placed by Blakely in the (1935) first edition.

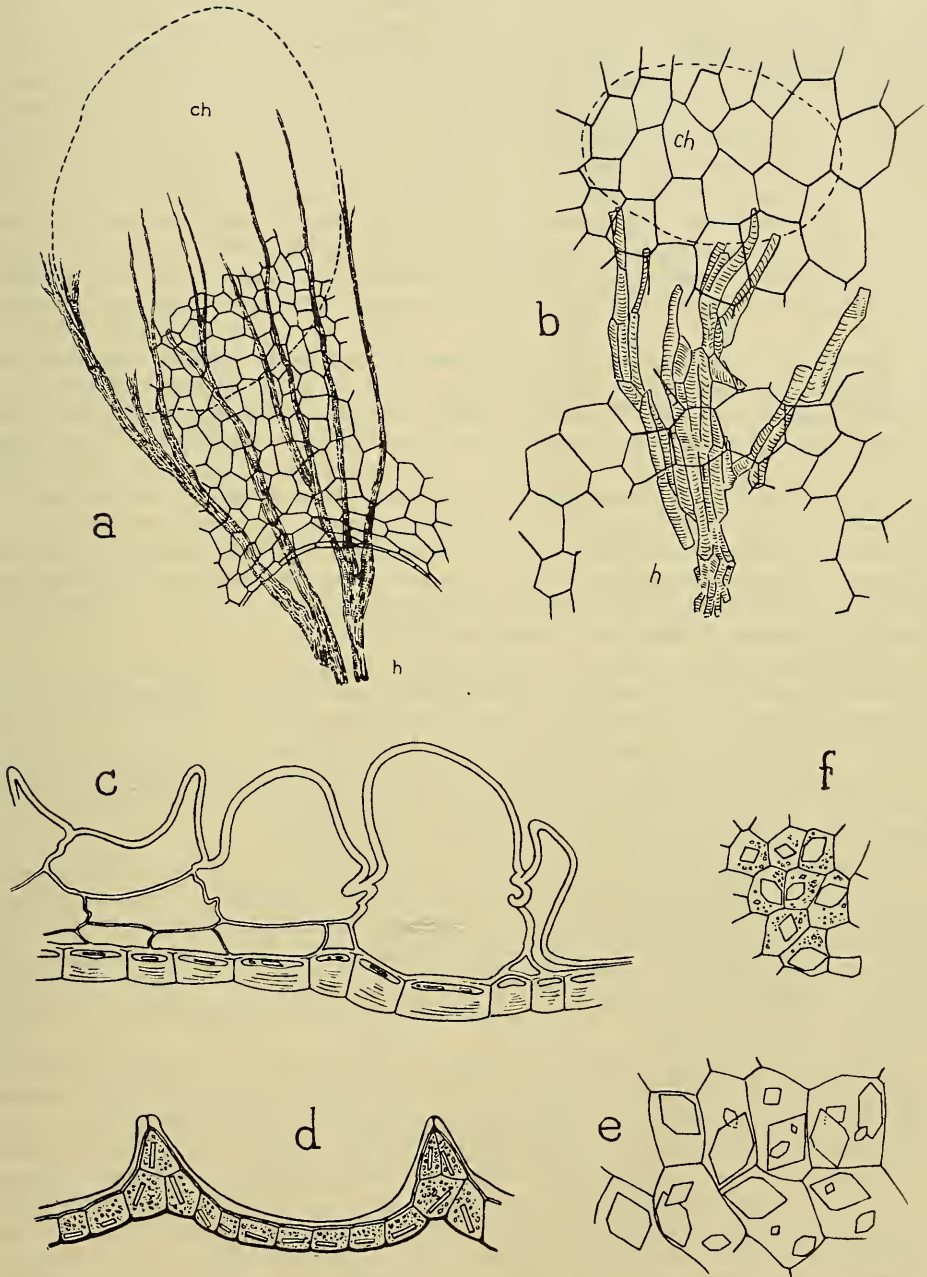
The three species *E. tetraptera*, *E. steedmanii* and *E. erythrandra* are dealt with together and at the same time the relationship to *E. forrestiana* is particularly considered.

The seeds are hemitropous, but in contrast with Eudesmiae, Miniatae, Clavigerae and Corymbosae the hilum is some distance below the chalaza, therefore the vascular bundle has to run to some extent through the outer integument, forming a short raphe, before reaching the chalaza (Fig. 41a). The outer cuticle is missing, the tender inner integument is not suberized. The chalaza cork is always present and its middle lamellae are lignified. In view of the tenderness of this tissue the limit between the middle lamella *sensu stricto* (that is, the intercellular substance alone) and the primary wall is obscure and it may be that the lignification affects both. The suberin pellicle forming the secondary wall can be isolated easily and stained.

This type of suberization combined with lignification was called by A. Meyer and his students "Metakutisierung" (metacutization) (see H. Müller, 1906; also E. Gauba, 1926).

Remnants of the nucellar tissue (the cells empty and obliterated) are present, often amounting to a considerable quantity. A striking feature is the extensive wall cutinization of its outer layers, especially in the proximity of the chalaza (Pl. iii, fig. 1). Fatty substances synthesized and released by the protoplasts of these cells accumulate not only on the surface of the nucellus to form finally a cuticle (coalescing with that of the inner integument), but also impregnate the periclinal and anticlinal walls up to six or more layers deep. This intramembranous cutinization is more or less continuous only in the outermost layers. Seen in section it is gradually reduced further into broken lines, granules and dots, which are more and more widely spaced the more distant they are from the surface (atypical cutinization). If we interpret this teleologically we could consider it as a substitute for the unsuberized inner integument (suberized in





Text-fig. 41a-f.—a, b, vascularization patterns: a, *E. tetraptera*, 75 $\times$ ; b, *E. steedmanii*, 220 $\times$ . c, d, outer integument in trans. sect., 350 $\times$ : c, *E. tetraptera*; d, *E. steedmanii*. e, f, crystal layer in surface view, 350 $\times$ : e, *E. tetraptera*; f, *E. steedmanii*.

Eudesmiae, Miniatae, Renantherae). Thus, having fulfilled its role as a nutritive tissue, the nucellus is converted to a tissue protecting the embryo.

The (black) seeds of *E. tetraptera* and *E. erythrandra*\* are morphologically identical and differ (in our sample) only in the size. There is a narrow circumferential wing, built up by the two integuments, and a system of folds (or narrow wings) on the ventral side, most of them radiating towards the raised hilum-chalaza region. However, Gardner (1940-41) considers *E. erythrandra* as a hybrid between *E. tetraptera* and *E. angulosa*. From our seed investigation it can be anticipated that no evidence of such a hybrid origin can be expected, because the seed structure of the putative parents is very similar in the salient characters.

On the other hand, Blakely (Key, p. 74-75), though listing *E. forrestiana* in the Section Platytherae, Series Contortae, has pointed to some affinities with the Tetrapterae. This suggestion is supported by the conformity of their external and anatomical seed structure. The same set of anatomical criteria is also encountered in *E. stoatei*. The suggestion by L. A. S. Johnson (*in litt.*)—on other grounds than seed structure—of a close relationship with *E. tetraptera*, has support also from our investigation. *E. steedmanii*† is an exception because of its entirely different outer integument. There is no testa expansion into wings or folds and the surface is finely honeycombed by transversely elongated pits in longitudinal alignment on the back and in rows radiating towards the hilum on the ventral side. This sculpture is due to the sunken outer walls of the large epidermal cells and (in cross section) to the undulate course of the underlying crystal layer. This type of ornamentation is encountered—as a test at random showed—in several Sections, e.g., *E. spathulata*, *E. torquata*, *E. intertexta* (Section Macrantherae), in *E. sideroxyylon* (Section Terminales) and in *E. viridis* (Section Porantheroideae). Further examples are given by Grose and Zimmer (1958) and illustrated in surface view in their fig. X on page 7. Our Figure 41d shows the anatomical structure in sectional view.

It is also worth mentioning that in *E. tetraptera* and *E. erythrandra* each cell of the crystal layer contains besides one large ensheathed crystal a few smaller ones, all geometrically well shaped (Fig. 41e), whereas in *E. steedmanii* there is only one large crystal surrounded by a considerable number of crypto-crystalline granules (Fig. 41f).

Another fact suggesting the exclusion of *E. steedmanii* from the Tetrapterae is the shape of the cotyledons, which are reniform in *E. tetraptera* and *E. erythrandra*, but—as a germination test showed—deeply bisected in *E. steedmanii*.

It is interesting that C. A. Gardner (*in litt.*), using other evidence, quite independently from our investigation, arrived at the same conclusion. In his opinion *E. steedmanii* should be placed in the Series Cornutae, close to *E. spathulata*. We examined *E. spathulata* and the seed structure agrees precisely with that of *E. steedmanii*, even with regard to seemingly trifling characters, as, for instance, the occurrence of crystal idioblasts (with crystal plus granules) in the integumentary parenchyma of the ventral side.

There is still another problem with regard to the position of the Series Tetrapterae. Blakely puts it between the Eudesmiae and Miniatae on the one side, and Clavigerae and Corymbosae on the other. These four Series have, contrary to the Tetrapterae, no raphe at all and therefore an entirely different vascularization pattern. This could be considered as a minor character, but it is significant that the seed of the Xylocarpae (*E. macrocarpa*, *E. pachyphylla*, *E. pyriformis*) agrees well in all salient characters (wings, short raphe, unsuberized inner integument, heavy cutinization of the nucellus) with the Tetrapterae, though the cotyledon shape is quite different: reniform in the Tetrapterae and bisected in the Xylocarpae. Of course, there is—as with the widespread

\* The seed was obtained through E. F. Martin and was collected from a cultivated tree at Bulgunnia Station homestead, 50 miles west of Mt. Eba (South Australia), by T. Gray in October, 1957.

† Authentic seeds of *E. steedmanii* were received from C. A. Gardner, Government Botanist, Perth.

occurrence of honeycombed seeds—always the question as to which characters arise independently in different unrelated groups, and which are phyletic, expressing true affinities. More facts are still needed before a definite answer can be given.

Series CLAVIGERAE (Maiden) S. T. Blake.

S. T. Blake separates seven species from Blakely's *Corymbosae*, uniting them into the Series *Clavigerae*. Most of them occur in relatively remote parts of northern Australia, and this and the fact that they shed seeds quickly after ripening accounts for the difficulty in obtaining sufficient quantities for examination. We have had a good quantity only of *E. papuana*, *E. tessellaris* and *E. confertiflora*, whereas the results of three other species, *E. clavigera* (Darwin, 1.12.1915, Hill 364), *E. grandifolia* (Burrundie, N.T., 5.11.1915, Jensen 352) and *E. aspera* (Roy Hill, W.A., 8.5.1958, Burbidge 6043), are based on one or two seeds obtained from authentic dried specimens. It is plain in these latter cases the examination could not be extended to all anatomical and histochemical details; nevertheless from those examined in detail and because of the consistency of the group we believe we have obtained an adequately accurate picture of the seed structure in this Series.

The seeds are hemitropous, strongly compressed dorsiventrally and therefore thin, flat or concave-convex, orbicular or ovate in outline, and with a narrow firm or tender circumferential border. From the point of view of dissemination these seeds would be called in German terminology "Scheibenflieger" (when orbicular) or "Scheibendrehflieger" (when ovate).

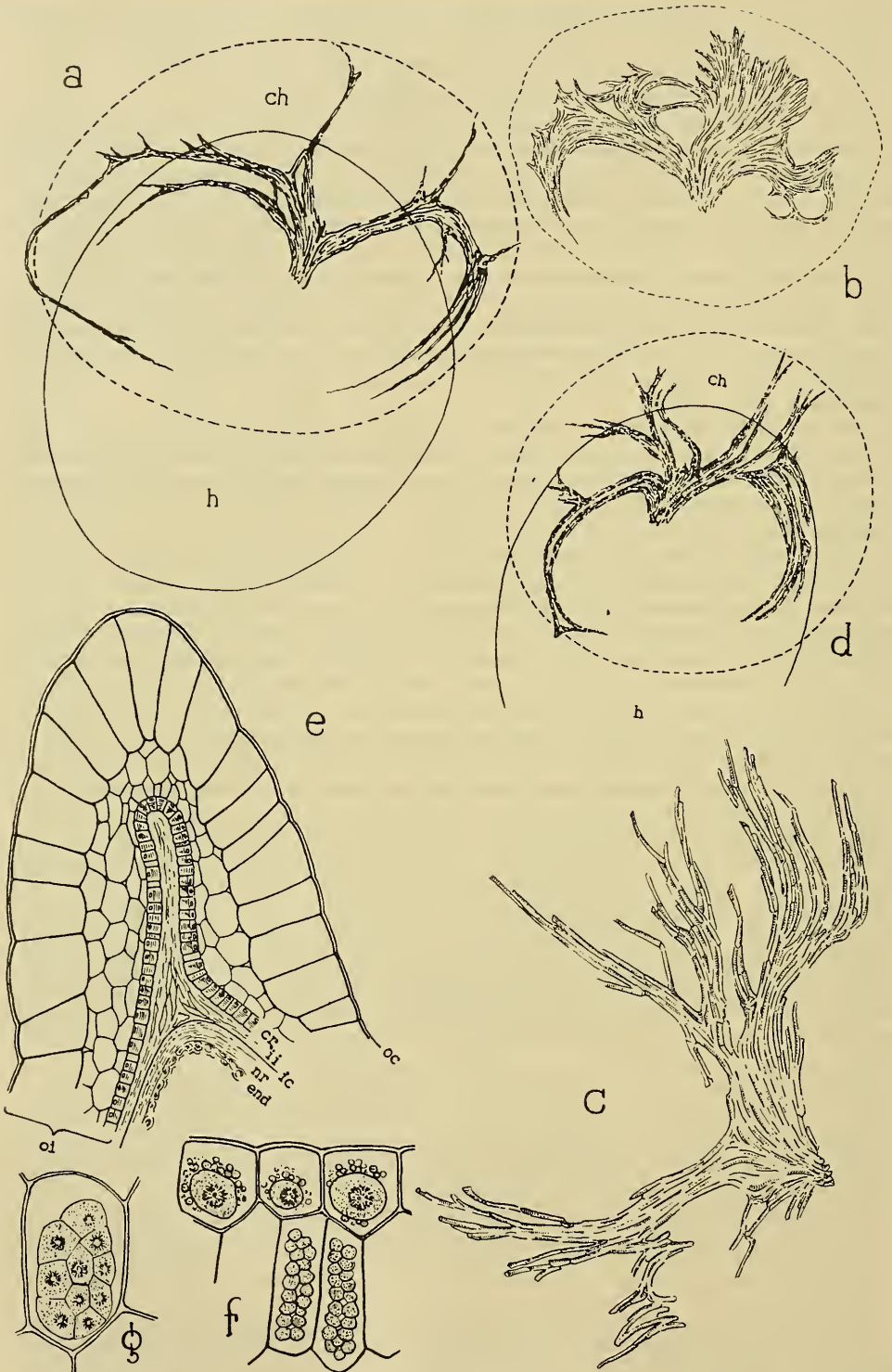
The outer integument is multilayered on the ventral side, but on the back reduced to three and here and there to two layers only (Fig. 43*d*). The outer cuticle is well preserved. The epidermal cells are thin walled, but some with thicker walls may be interspersed (*E. papuana*, *E. confertiflora*). They contain either amorphous reddish-brown deposits or are filled with a yellowish or reddish solution which is precipitated by potassium dichromate, copper acetate or formalin. Rhodamin gives a blue flocculent precipitate, ferric salts stain the contents dirty green or blue, and osmic acid inky blue to black. These reactions point to tannins. Both solution and solid deposits assume a brilliant ruby-red colour with vanillin-HCl, indicating the presence of a phenol, very probably of phloroglucin, and may be considered as phloroglycotannoids. In *E. papuana* most of the epidermal cells are filled with a solution, easily extracted by water or alcohol, and a few only have solid deposits. The latter were the only type seen in *E. confertiflora*.\* The proportion of cells containing solution to those with solid deposits varies within the species and generally it can be said that the greater the amount of these deposits the darker the seed colour, which varies from light brown to black. In *E. papuana* the small epidermal cells surrounding the exostome are crystal-bearing, the walls being either thin (Fig. 43*a*) or sclerified to such a degree that the crystals become embedded in the wall thickenings (Fig. 43*b*). This encasement can be complete, but more frequently the uneven wall thickenings form only a "pocket" around the crystal. Besides this ergastic envelope the crystals have also their own alloplasmic sheath originating probably from a cellulosic degeneration of the protoplast (Fig. 43*c*). Mechanical elements in the integumentary parenchyma are rather rare, but can be seen for instance beneath the hilum scar in *E. papuana*. Sclereids forming an uninterrupted layer between the two epiderms on the dorsal side were seen only in *E. aspera*.† The inner epidermis is a typical crystal layer with thick outer walls (crystal sclerenchyma), leaving but little space for the crystals.

The inner integument is tender, not suberized and here and there resorbed. The inner cuticle was seen to be almost smooth in *E. clavigera* and *E. confertiflora*, with

\* These deposits were still undissolved in sections kept for one month in water and in alcohol.

† We had only one seed for examination. Though the embryo was apparently of normal structure, some anomalies in shape and size of the seed and its venation pattern give rise to some doubt whether this occurrence of sclereids is a peculiar and constant character in this species. Heavy sclerifications in the testa are common in seed which is sterile or inviable for some reason or other.





Text-fig. 42a-g.—a, b, c, d, vascularization patterns: a, *E. papuana*, 35×; b, *E. clavigera*, 23×; c, *E. confertiflora*, 60×; d, *Angophora intermedia*, 35×. e, trans. sect. through the circumferential border of *E. papuana*, 150×. f, g, oxalate druses in the aleuron grains of the embryo cotyledons, 730×: f, in the upper epidermis of *E. papuana*; g, in a mesophyll cell of *E. tetraptera*.

short ribs in *E. papuana* and with few but very long projections (up to eight layers deep) into the radial walls of the nucellus in *E. tessellaris* and *E. grandifolia* (see Pl. iii, fig. 5, of *E. gummifera*).

The circumferential border of the seed is built up by both integuments and is relatively thick in *E. papuana* (Fig. 42e) and *E. confertiflora*, but very thin in *E. tessellaris*. In *E. grandifolia* we saw also the inner cuticle, the nucellar tissue and even some endosperm penetrating to some extent into the border.

The *hilum* overlaps the chalaza and the vascular bundle enters directly into it, whereupon it ramifies, forming discrete strands of variable thickness, the two outermost the strongest, and with an arcuate course along the chalaza border (*E. papuana*, Fig. 42a; *E. grandifolia*). In *E. confertiflora* the strands are very loose and their arrangement varies. They are turned upwards (like antlers, Fig. 42c), or spread horizontally or the outermost bent down. In the two seeds of *E. clavigera* examined (Darwin, 1.12.1915, Hill 364) the tracheids were flatly spread, like a fan, covering a great portion of the chalaza (Fig. 42b). Commonly one single bundle enters the chalaza, but in *E. confertiflora* we saw sometimes two and even three separate bundles.

The *chalaza cork* has lignified middle lamellae and is filled with dark tanniferous deposits. This seems to be a general rule for the whole genus, only very few exceptions having been observed hitherto. In *E. confertiflora* (from Port Moresby, Papua) most of the seed (but not all) have a considerable amount of a mineral deposit in the chalaza parenchyma around the tracheids. There are granules, loose or aggregated into grape-like clusters, crystals, partly or completely corroded, but well-shaped intact crystals of tetragonal bipyramids occur too. Sulphuric acid converts them all on the spot in clusters of needle-shaped crystals of calcium sulphate. Consequently they belong to the trihydrate of calcium oxalate whose crystals belong to the tetragonal system. It is interesting to see both hydrates in adjacent tissues: the monoclinic monohydrate in the crystal layer, the tetragonal trihydrate in the chalaza.

Remnants of nucellus and endosperm are present, the former multilayered, the latter mostly as a single strongly compressed layer.

Morphologically and anatomically the seeds of the Clavigerae agree in all essential characters with those of *Angophora*. There is also the same occurrence of amorphous and dissolved phloroglycotannoids in the epidermal cells. A further remarkable feature relates to the location of cluster crystals (druses) of calcium oxalate in embryo cotyledons. In the Eudesmieae, Miniatae and Tetrapterae they are confined to mesophyll cells (Fig. 42g). In the Clavigerae (and as will be shown later in the Corymbosae too) they are found exclusively in the epidermis. Here each cell contains some small aleurone grains (without any inclusion) surrounding one very large "solitaire" in which one druse of considerable size is embedded (Fig. 42f). This applies only to the upper epidermis, whereas in the lower the occurrence of very small druses is not so constant. The Clavigerae and Corymbosae have this peculiar distribution of cluster crystals in common with *Angophora* (*A. intermedia*, *A. cordifolia*, *A. costata*).

The above results show in review that the seed structure in its essential traits supports the opinion that the Series Clavigerae, as formulated by Blake, is a natural one.

#### Series CORYMBOSAE (Benth.) Maiden. (23 species examined.)

Seed hemitropous, compressed either dorsiventrally and then wingless, or laterally when (with very few exceptions) it is furnished with a long terminal wing.

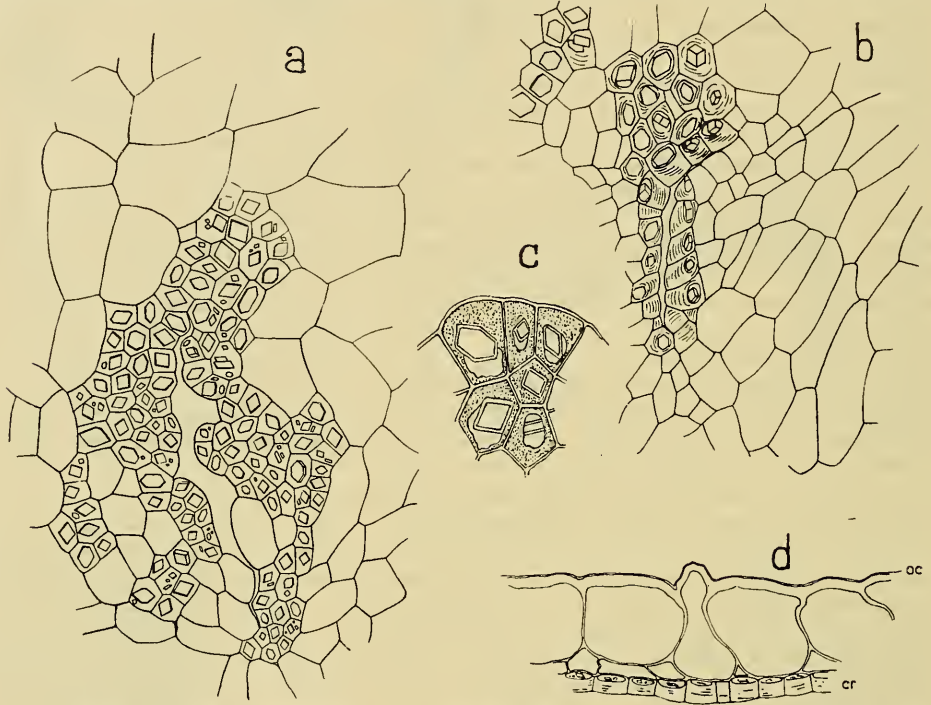
The *outer integument* is multilayered, though in some species the epidermis on the dorsal side may lie directly upon the crystal layer (e.g., *E. trachyphloia*, *E. jacobsoniana*). Where the surface is wrinkled, the outer integument appears in cross sections more or less deeply lobed, e.g., *E. abbreviata* (Fig. 44c), *E. foelscheana*, *E. latifolia*.

The outer cuticle is always well preserved.

The epidermal cells are rectangular in cross section, somewhat perpendicularly elongated or even palisade-like towards the edges (Subseries Maculatae), and then some of them are horizontally divided. The outer walls are commonly rather thicker and some of them lignified (e.g., in *E. setosa*, *E. terminalis*, *E. ficifolia*, *E. bleeseri*).



In most of the Setosae and Eucorymbosae the epidermal cells have (like some sub-epidermal cells) uneven thickenings on the inner and side walls (Fig. 44*b*). They are all filled with light or dark reddish-brown tanniniferous substances, either dissolved or as amorphous deposits, often glasslike (Ochrophloiae, Maculatae), and either readily or scarcely soluble in water or alcohol.\*  $\text{FeSO}_4$  stains inky blue, vanillin-HCl ruby red. They may be classified as phloroglycotannoids. The conspicuous deposits on the outer walls of *E. calophylla* (Fig. 44*a*) deserve particular consideration. They are unique among the Corymbosae. Petit has recorded and illustrated them in his Fig. 19 on page 48. He mentions their mucilaginous aspect and noticed also a brownish substance



Text-fig. 43*a-d*: *E. papuana*.—*a*, *b*, *c*, crystal-bearing epidermal cells around the open exostome in surface view: *a*, the cells thin walled, 175 $\times$ ; *b*, the cells sclerified, 230 $\times$ ; *c*, a group of them after treatment with HCl to remove the crystals, 350 $\times$ ; their shape is preserved perfectly by their alloplasmic envelope which is embraced by the (stippled) secondary uneven wall thickenings (ergastic envelope). *d*, trans. sect. through the outer integument, dorsal side, 230 $\times$ .

in the lumen. Netolitzky (p. 240) suggests that the wall deposits are probably the so-called "inclusions". We examined seed gathered ourselves from a natural stand (Dwellingup, W.A.) with the following results:

Cross sections show considerable deposits on a thick outer wall and part of the side walls, adhering firmly to the walls and occupying about one-third to one-half of the cell cavity (Fig. 44*a*). After swelling is induced a fine and dense lamellation is revealed together with simple or ramified pit canals. In very thin sections the outer layers appear nearly colourless, but, due to a gradually intensified impregnation with dark substances, the colour of the subsequent layers changes from light brown to almost black in the innermost. Chloroidide of zinc stains the light lamellae pure blue, the heavily impregnated inner ones dirty blue. IKI (iodine + potassium iodide) alone does not stain them, but the addition of sulphuric acid produces under strong swelling a dark to blackish-blue staining. Cuprammonia dissolves them. Boiling in 2 per cent.

\* In sections of *E. foelscheana*, kept for a month in water and in alcohol, most of the deposits were still undissolved.

HCl for two hours causes no hydrolysis. All these reactions point to cellulose as the ground substance.

The impregnating substance responds to tannin reagents. In very fine sections the nearly colourless outer lamellae remain unstained with  $\text{FeSO}_4$ , the brownish inner ones become greenish or olive-green. The three reagents considered as reliable indicators of phloroglycotannoids (which are characteristic for the "inclusions"), vanillin-HCl, p-dimethylaminobenzaldehyde- $\text{H}_2\text{SO}_4$ , and KOH, have no effect except for some bleaching with KOH. It is concluded from the above reactions that the wall deposits have a cellulosic ground substance impregnated with tannins but not with phloroglycotannoids. In water these deposits show no noticeable swelling,\* but after treatment with Eau de Javelle (to remove the tannins), followed by washing with water, they swell markedly, filling the whole cavity. Alcohol contracts these masses to their original volume. We have already encountered the same wall deposits—though less conspicuously—in the Renantherae, Series Occidentales (*E. marginata*, *E. staerii*, *E. sepulcratis*) and have considered them as membrane-mucilage impregnated with tannins.

The chemical behaviour of the yellowish or brick-red material secreted in the cavity of the epidermal cells is quite different. It is also present in all parenchyma cells of the outer integument. Vanillin-HCl or p-dimethylaminobenzaldehyde- $\text{H}_2\text{SO}_4$  gives a brilliant ruby-red colour constantly.

The impregnated parenchyma walls are also stained pink or red, while  $\text{FeSO}_4$  stains (in thin sections) these walls and the contents pure inky blue, often with a tinge of violet. Here we are dealing obviously with the same tannin derivatives, the phloroglycotannoids, we have already encountered in the Clavigerae.

*Integumentary parenchyma* is quite generally initiated by divisions in both epidermal layers (predominantly in the outer one), though when fully developed this origin is seldom recognizable. Only in some sections of *E. ptychocarpa* the three outermost layers demonstrated clearly their formation from the outer epidermis by periclinal and anticlinal partitions (Fig. 44b). Phloroglycotannoids as deposits and wall impregnation are very common. Sclereid idioblasts were seen in *E. gummifera* and *E. terminalis*.

*Crystal layer.* Various deviations from the typical structure were occasionally observed.

(1) It is, for instance, not unusual that cells of this layer at the chalaza border are without crystals.

(2) Patches of empty cells within this layer are a regular feature in *E. trachyphloia* (from Belmont, Brisbane) and also frequent in *E. ptychocarpa*.

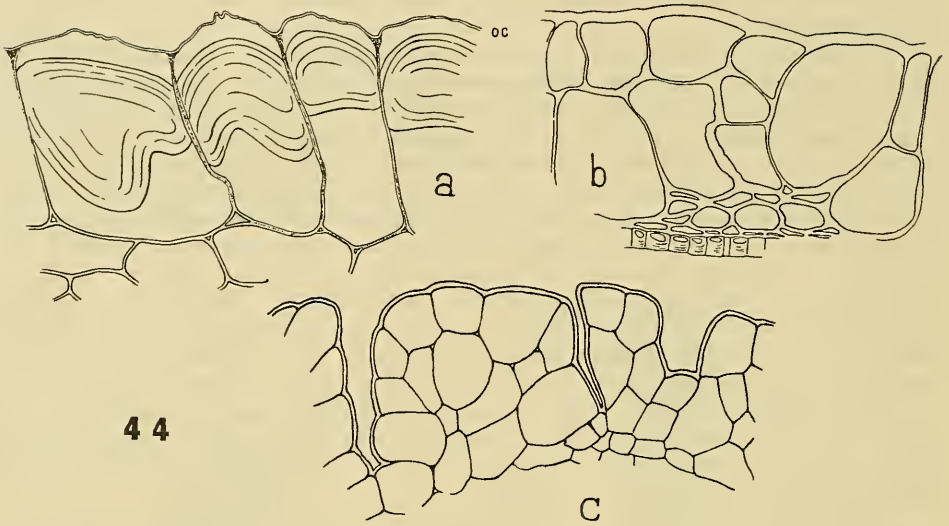
(3) In *E. haematowylon* and *E. maculata* the crystal layer is occasionally infolded against itself at the rim of the chalaza so as to reverse the orientation of the crystal-bearing cells (Fig. 45a). As previously mentioned, this is quite common in *E. phoenicea*.

(4) An occasional doubling of the crystal layer in patches anywhere along its course may occur, the additional layer being adjacent to the outer face of the basic crystal layer. The crystal-bearing cells may have the same orientation (*E. latifolia*, *E. abbreviata*, Fig. 45b), or are inverted (*E. ferruginea*, Fig. 45c).

The *inner integument* in many species is in some places more or less resorbed and—except at its base on the chalaza border—not suberized. The inner cuticle (coalescent with that of the nucellus) is always present, either (in cross sections) with short ribs or, especially near the chalaza, with long projections penetrating sometimes up to twelve layers deep into the anticlinal walls of the nucellus (Pl. iii, fig. 5). In longitudinal sections they appear as narrow wavy strips quickly decreasing in width.

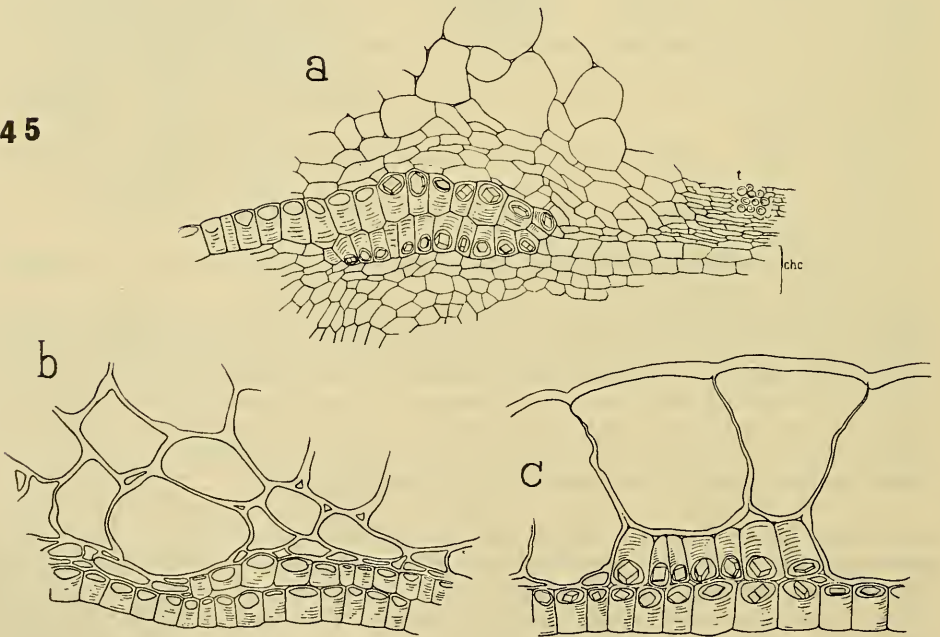
*Wings.* The presence of a long terminal wing is characteristic of certain groups of the Corymbosae and unique in the genus. This wing, usually about as long as the body, is the result of an extension of the outer integument, but without the participation of the crystal layer, and causes the seed to move spirally during dissemination ("Schraubenvlieger"). Its formation is strictly confined to species with seed flattened or

\* Mucilages are known which scarcely swell in water, e.g., that of *Cinnamomum cassia*.



44

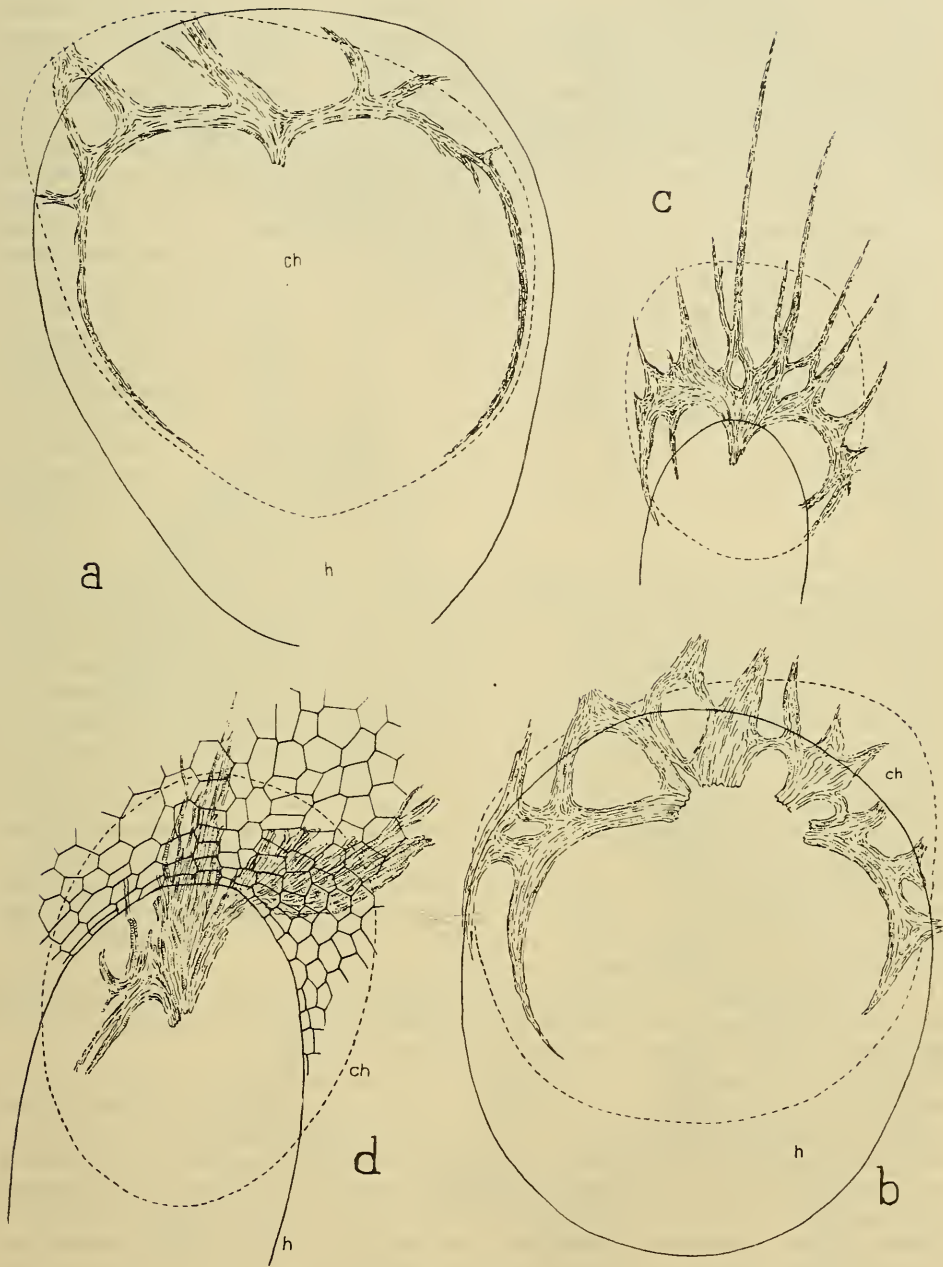
45



Text-fig. 44a-c.—Part of outer integument in trans. sect.: *a*, *E. calophylla*, 230 $\times$ , epidermal cells with wall deposits; *b*, *E. ptychocarpa*, 150 $\times$ , showing origin of some integumentary parenchyma from the outer epidermis by periclinal and anticlinal divisions; *c*, *E. abbreviata*, 90 $\times$ , outer integument lobed.

Text-fig. 45a-c.—Occasional doubling of the crystal layer, 230 $\times$ : *a*, *E. haematoxylon*, crystal layer infolded against itself at the rim of the chalaza; *b*, *E. abbreviata*, the additional layer (crystals omitted) with the same orientation; *c*, *E. ferruginea*, the short additional layer with inverse orientation of the crystal-bearing cells.





Text-fig. 46a-d.—Vascularization patterns: a, *E. calophylla*, 15 $\times$ ; b, *E. haematoxylon*, 15 $\times$ ; c, *E. abbreviata*, 23 $\times$ ; d, *E. dichromophloia*, 75 $\times$ .

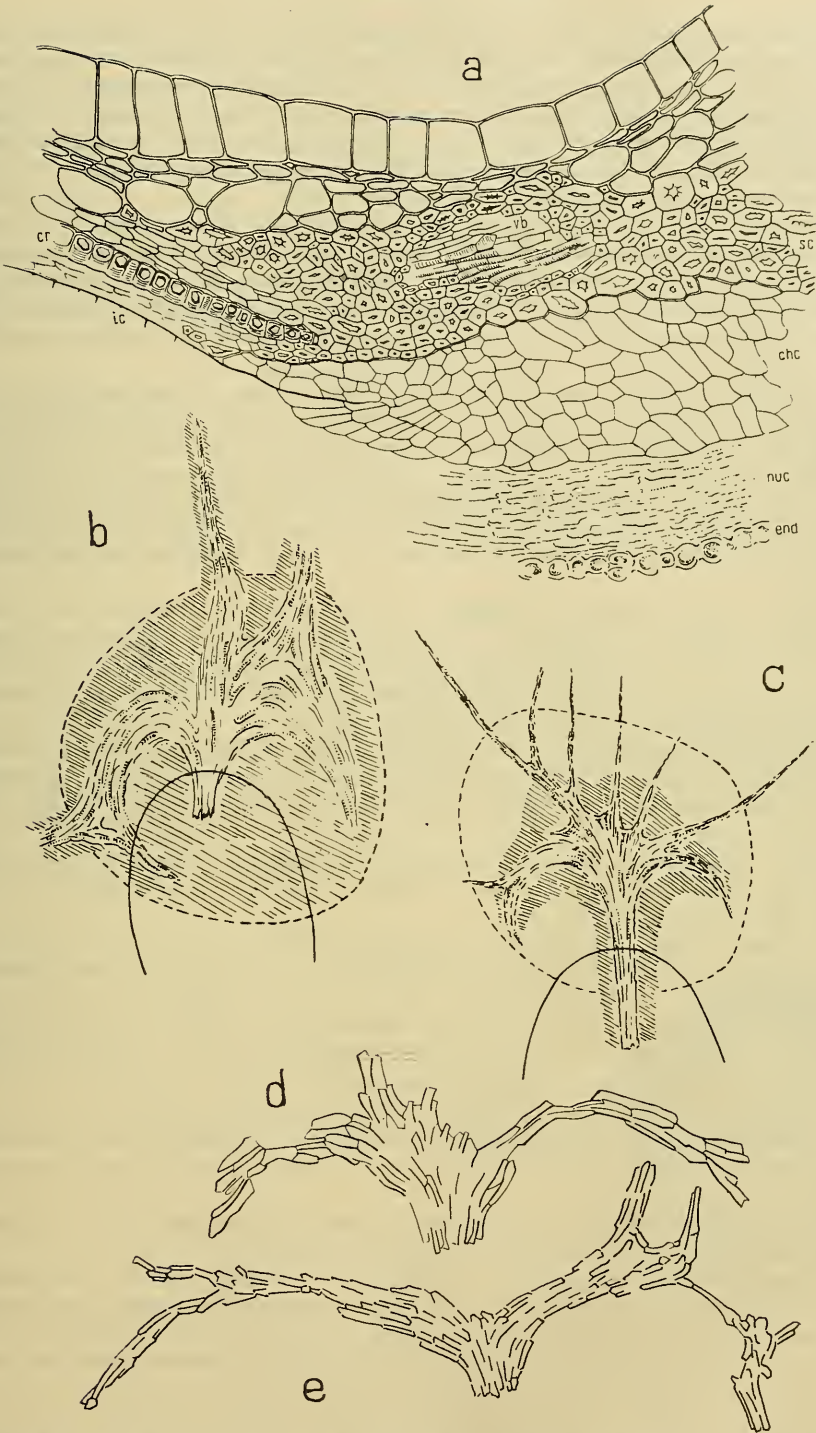
compressed laterally, thus excluding the Subseries Ochrophloiae and Maculatae which have dorsiventrally flattened wingless seed. On the winged seeds the hilum and chalaza are located on one flank more or less close to the inner edge.\* The wing shape varies sometimes within the species, is frequently flat and ligulate, sometimes spoon-like or even boat-shaped. If the seed is very strongly compressed so that hilum and chalaza are lodged on the narrow inner edge, then the wing is folded. Adopting some of S. T. Blake's adjustments to Blakely's grouping we see that all species belonging to the Setosae (3), Gamophyllae (1) and Eucorymbosae (6) are furnished with a wing (*E. collina* is considered by Blake of doubtful validity as a species). In the Neocorymbosae (12) six species have a long terminal wing, in *E. gummiifera* it is small, in *E. haematoxylon*—if present—rudimentary, and it is missing in *E. calophylla*, but they all have laterally compressed seed. *E. trachyphloia* is out of step in this Subseries, its wingless seed being dorsiventrally flattened. Thus, whilst in the first three Subseries of the Corymbosae all species have winged seeds, the fourth includes a few with missing or imperfect wings. Bentham (III, 1888) has already pointed out that this character appears to be of little value if we consider, for instance, the two allied species *E. calophylla* and *E. ficifolia*. These are closely similar in every other respect, differing from each other mainly in their wingless or winged seeds. In our opinion it is not so much the wing but the shape of the seed body (flattened laterally or dorsiventrally) which, apart from other characters, expresses affinity or lack of it in these species. If this character is of taxonomic value then it supports a readjustment in the placing of *E. trachyphloia*. It is noteworthy that Jacobs (1935) suggested some affinity with *E. jacobsiana* placed by Blakely in the Subseries Ochrophloiae of which all species have dorsiventrally flattened seed.

The *chalaza* accommodates the conducting tissue, of which some patterns (though at times variable even in the same species) are illustrated in Figures 46 and 47. The poor vascularization in *E. trachyphloia* (Fig. 47*d*) is remarkable and reminiscent of that in *E. jacobsiana* (Fig. 47*e*, type material), consisting mainly of two more or less curved branches. Of course, this poor vascular supply may be due to the size of the seeds (3–3½ mm.) which are the smallest among the Corymbosae. Nevertheless, the similarity of the patterns could perhaps also lend some support for the transfer of *E. trachyphloia* from the Neocorymbosae to the Ochrophloiae. With regard to the relative position of xylem and phloem a few observations of cross sections may be mentioned. In *E. ficifolia* and *E. calophylla* amphicribal bundles were seen sometimes, but as a rule collateral bundles are prevalent with the phloem on the outside of xylem (*E. latifolia*, *E. ptychocarpa*, *E. foelscheana*) and in *E. setosa* occasionally on its inside. In *E. watsoniana* and *E. setosa* the phloem was sometimes in a position lateral to the xylem. These differences are probably due to a variation in the arrangement of the two bundle components in different levels of its course, from the large still undivided bundle at its base to the terminal veinlets.

In many species of the Neo- and Eucorymbosae the conducting tissue is embedded in a small-celled, tannin-impregnated tissue, obliterated beyond recognition. In the Maculatae it runs in a solid, thick plate of sclereids (Fig. 47*a, b*).

Occurrence of sclereids in the chalaza is often encountered. They are either dispersed or forming a more or less closed layer of variable thickness, adjacent to the chalaza cork and beneath the vascular system. In *E. calophylla* this layer is only one to two cells thick, in *E. ferruginea* four to seven. Their occurrence in *E. trachyphloia* is not constant. In seeds we collected at Belmont (Brisbane) the sclereids are abundant and dispersed all over the chalaza, but quite absent in seeds from Beerburum. A gradual increase of sclereids from species to species can be observed in the Ochrophloiae.

\* In the description of the winged Corymbosae Maiden (VII, 105 ff.) comments on the position of the hilum in relation to the *endosperm* (!), e.g., "hilum about the centre of the endosperm", "hilum on the upper half of the endosperm", etc. Now, the hilum is a scar on the testa which envelops nucellus, endosperm and embryo. The nucellus is multilayered and the endosperm generally a single layer of cells. They both extend completely around the inner side of the testa and surround the embryo. What Maiden has in mind is undoubtedly the proper *seed body* (without the wing) and not the *endosperm*.



Text-fig. 47.—*a*, *E. maculata*, trans. sect. through the chalaza region, 230 $\times$ . (Bundle strand *vb* embedded in the sclerenchyma plate *sc*.) *b-e*, vascularization patterns: *b*, *E. maculata*, 35 $\times$ ; *c*, *E. watsoniana*, 35 $\times$ ; *d*, *E. trachyphloea*, 175 $\times$ ; *e*, *E. jacobsiana*, 175 $\times$ .

The shaded parts in *b* and *c* show the extent of sclerification around the conducting tissue. The broken line limits the chalaza.



*E. bloxsomei* is devoid of them. In *E. torrelliana* and *E. eximia* conspicuously pitted sclereids are loosely arranged, singly or in groups, in the lower part of the chalaza. In *E. peltata* and *E. watsoniana* (Fig. 47c) they form a solid layer one to three cells thick. A further step leads to the Subseries Maculatae where the highest degree of sclerification is achieved. Here, the mechanical tissue extends over the whole chalaza, embedding completely, as just mentioned, the conducting tissue and accompanying the bundles to their very ends beyond the chalaza (Fig. 47a, b).

All these mechanical elements are formations of the chalazal tissue. However, in *E. watsoniana* there are also sclereids as part of the conducting tissue. In cross sections through the lower still undivided part of the bundle the xylem is surrounded by a crescent-shaped plate of sclereids which on the phloem side are only scattered. The fact that these mechanical cells can be already discerned on the bundle stump protruding from the hilum is evidence enough for their origin.

The closure of the chalaza (on its inside) is as a rule accomplished by a tender lignified and suberized tissue, filled with tanniferous deposits staining ruby-red with vanillin-HCl. As previously mentioned, the designation of this tissue as "chalaza cork" is not quite appropriate. Using this term is only a matter of convenience. It is not a cork in the sense of the phellem originating from a phellogen. It is not exclusively derived from chalazal tissue, because nucellar tissue too can take part in its formation; finally it may not be suberized. These facts are best illustrated by the following examples.

A cross section through the chalaza cork of *E. calophylla* shows in its outer part thick-walled, pitted and lignified cells interspersed among the thin-walled suberized ones, thus somewhat reminiscent of the occurrence of sclereids within the phellem of some plant species. Here and there the lignified cells may be arranged more compactly along the periphery of the chalaza cork (Pl. iii, fig. 4).

In *E. haematoxylon* the outer part consists of lignified and suberized cells arranged in orderly parallel rows whilst the inner part consists of irregularly disposed suberized, but not lignified, nucellar cells passing at the circumferential chalaza border into merely cutinized nucellus cells.

Finally, we remember that in the Miniatae, for instance, there is neither suberization nor lignification in that part of the chalaza which in other species is generally occupied by a suberized tissue.

The hilum overlaps the chalaza to a lesser or greater degree. In *E. calophylla* and *E. haematoxylon* it is almost entirely so (Fig. 46a, b). Suberization was observed in *E. setosa* (Darwin, W. Bateman, Dec., 1957), but was sometimes only patchy and restricted to that part which overlaps the chalaza (Pl. iii, fig. 2). Small groups of suberized cells were also seen in *E. terminalis* (Ayers Rock, N.T., 4.6.56, N. Forde, 134).

*Nucellus and endosperm.* Remnants are always present, the former multilayered, the cells empty and obliterated, the latter generally as a single layer.

*Oxalate druses.* As in the Clavigerae and species of *Angophora*, they are confined to the epidermis of the embryo cotyledons and embedded only in the large aleurone grains.

The results obtained from this anatomical review of the testa of the Corymbosae support the erection of two groups on the basis of seed shape.

(1) Seed laterally flattened or compressed: Subseries Setosae, Gamophyllae Eucorymbosae and Neocorymbosae. This would roughly correspond with Maiden's "Terminaliptera", but including *E. calophylla* and *E. gummifera*. Nearly all the species have a long terminal wing which, however, is of restricted taxonomic value as revealed by the two closely related species *E. calophylla* and *E. ficifolia*.\* On the other hand the

\*The two species have nevertheless some other remarkable differences in their testa structures. The considerable wall deposits in the epidermal cells of *E. calophylla* are missing in *E. ficifolia* (as well as in all other Corymbosae). The chalaza cork of *E. ficifolia* is a uniform tender suberized tissue which in *E. calophylla* is, as already mentioned, interspersed with sclerified but not suberized cells—a structure not yet encountered among the species hitherto examined.

testa structure is of no help in providing confirmatory evidence for the separation of Blakely's Subseries based on morphological characters of other organs.

(2) Seed compressed dorsiventrally: Subseries Ochrophloiae and Maculatae. This would cover Maiden's "Naviculares" (except *E. calophylla* and *E. gummifera*). The seeds are wingless, but in some species bordered by a tender narrow membrane sometimes a little enlarged on the upper (or also lower) end of the seed. The Maculatae are undoubtedly a well defined group for which the testa structure too provides clear evidence, especially by the embedding of the vascular system in a thick plate of sclerenchyma tissue. This character links the Maculatae with the Ochrophloiae, where from species to species a gradual increase in the sclerification of the chalazal tissue can be observed, culminating in *E. watsoniana*, but without reaching the extent seen in *E. maculata* and *E. citriodora*.

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#### EXPLANATION OF PLATE III.

(Cork tissue, cuticles and cutinized walls brown.)

1, *E. tetraptera*, trans. sect. of the chalaza, 180x, showing the chalaza cork and the cutinization of the nucellus. 2, *E. setosa*, part of the trans. sect. through the hilum-chalaza region, 140x. 3, *E. miniata*, part of surface view of the hilum with two entering bundles and the sclereid rim, 90x. 4, *E. calophylla*, central part of the "chalaza cork" in trans. sect., 230x. The outer part lignified, the inner suberized. 5, *E. gummifera*, nucellus in trans. sect., 350x, showing the inner cuticle and the cutinized anticlinal walls.

